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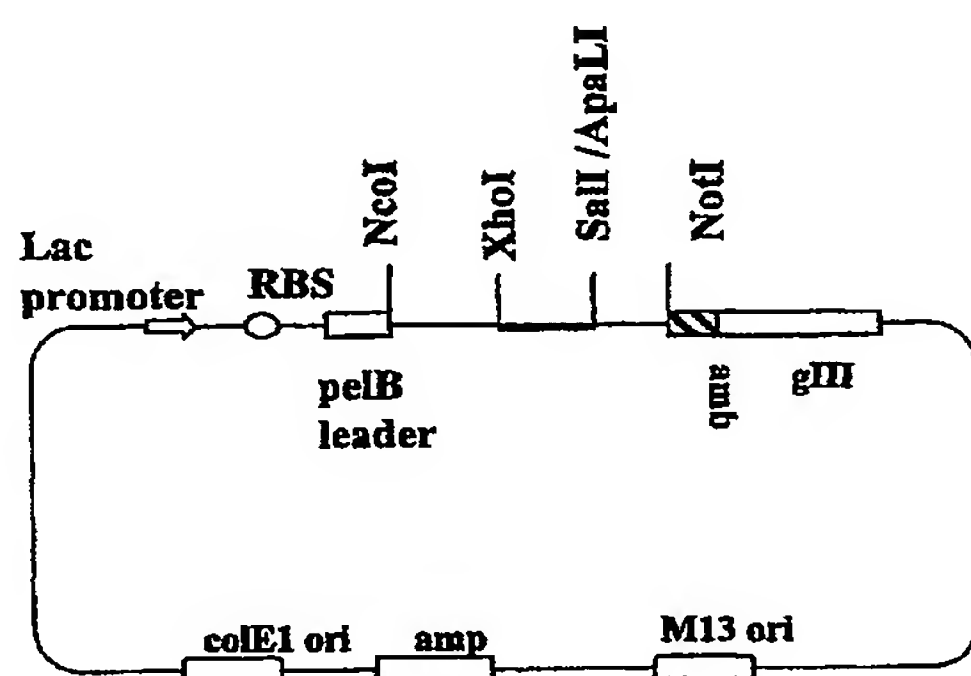
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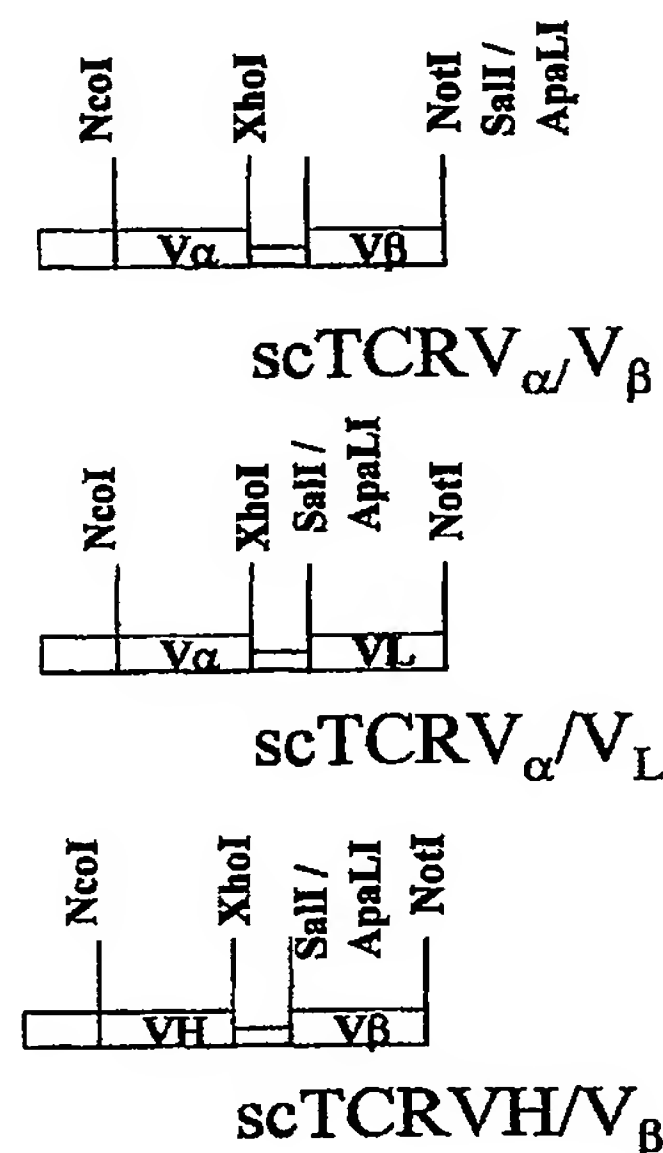
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(54) Title: CHIMERIC AND TCR PHAGE DISPLAY LIBRARIES, CHIMERIC AND TCR REAGENTS AND METHODS OF USE THEREOF



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B

(57) Abstract: This invention provides a chimeric phage display library, TCR phage display library, recombinant chimeric reagents, methods of making the libraries, methods for identifying recombinant reagents, oligonucleotides, linkers, tags, methods of purification, methods of increasing the avidity of recombinant reagents, methods of diagnosing and treating a subject with a disease or a pathogenic condition.

**CHIMERIC AND TCR PHAGE DISPLAY LIBRARIES, CHIMERIC AND TCR  
REAGENTS AND METHODS OF USE THEREOF**

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**FIELD OF THE INVENTION**

This invention provides a chimeric phage display library, TCR phage display library, recombinant chimeric reagents, methods of making the libraries, methods for identifying recombinant reagents, oligonucleotides, linkers, tags, methods of purification, methods of increasing the avidity of recombinant reagents, methods of diagnosing and treating a subject with a disease or a pathogenic condition.

**BACKGROUND OF THE INVENTION**

15 The immune system of vertebrates employs two principally different strategies to specifically combat foreign infectious agents or potentially harmful cells generated within the organism: the humoral and the cellular immune response. The essence of the humoral immune response is the mass production of antibodies (Ab) directed against the pathogen (typically, this will be of foreign origin), while within a cellular immune response, cytotoxic cells are generated having the ability to recognize and destroy pathogenic cells. Each of these two arms of the immune system is essential for its normal functioning. Disturbances of the balance between the two types of immune responses in humans leads to various types of diseases which are often life-threatening.

Although Ab, even within one organism, belong to diverse classes, they share a number of important features: they are typically soluble molecules, and consist of two types of chains, heavy (H) and light (L), which are constructed from structurally similar building blocks (domains). Furthermore, each chain consists of an N-terminal variable (V) domain, and a variable number of constant (C) domains. The V domains of H and L chains are designated  $V_H$  and  $V_L$ , respectively. In an intact Ab molecule, they are in close contact and are responsible for the antigen-binding property of the Ab. However, within a

given V domain, only certain sequences are actually contacting an antigen and exhibit extreme variability, the complementary determining regions (CDR). For each V domain, there are three CDR called CDR1, CDR2, and CDR3, respectively.

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Typically, the reactivity of an Ab is characterized by two important features: specificity and affinity. Because Ab consist of at least two identical antigen-binding sites (paratopes), it is possible that the overall affinity of an Ab molecule towards an antigen with multiple identical antigenic determinants (epitopes) must be defined by the combined affinities of the paratopes engaged in binding these epitopes. These combined affinities have also been referred to as avidity. This concept is particularly important in the case of IgM molecules, where the individual paratopes interact often only with low affinity. The avidity of the interaction is, nevertheless, high because an IgM molecule has theoretically ten antigen binding sites which could engage in parallel with a suitable antigen.

In theory, these properties should make Ab extremely versatile tools for diagnostic and therapeutic approaches. However, in the course of an immune response, an enormous variety of different Ab are produced, which makes standardization and continuous production of antisera with identical properties an impossible task. This principal difficulty has been solved by the invention of monoclonal antibodies (mAb)[1]. After immunization of an animal, typically a mouse, with an antigen, the Ab-producing cells are immortalized by fusing them with a myeloma cell line. A cell line secreting an Ab of the desired specificity can then be obtained using screening and cloning techniques. It has been possible to produce mAb against a wide variety of antigens, many of them also of diagnostic and therapeutic importance. However, mAb do have disadvantages, since their production relies on prior successful immunization. Another difficulty relates to their origin: mAb of animal origin are immunogenic after injection into humans. Furthermore, mAb have a molecular weight of at least 150.000, making penetration into solid tumours relatively inefficient [2]. Some, but not all, of these difficulties could be circumvented if mAb of human origin were available. However, even in transgenic producing human Ab [3],

prior immunization is absolutely essential, thus limiting the general applicability of this technology.

The display of repertoires of Ab fragments on the surface of filamentous bacteriophage offers a new way of making mAb-like reagents, bypassing myeloma hybrid technology and even immunization [4-10]. Recombinant Ab fragments of human origin have been isolated not only with specificities against both foreign and self antigens, but with specificities that are difficult to make by the conventional mAb technology. The recombinant reagents are either expressed as single chain (sc) Fv fragments ( $V_H$  and  $V_L$  joined by a suitable linker) or Fab fragments ( $V_H$  and  $V_L$  joined to their respective constant domain fragments  $C_{H1}$  and  $C_L$ ). These reagents are considerably smaller than Ab, likely to be less immunogenic in humans than conventional mAb, and their production does not require any immunization and is usually rapidly achieved.

The recombinant reagents are initially displayed as fusion proteins with a minor coat protein of filamentous phage particles. Each phage particle carries the genetic information for the recombinant reagent that it displays on its surface. This feature allows to identify the genetic information encoding an scFv fragment exhibiting a particular specificity by selecting that phage particle which carries it from a potentially very complex phage library. Since it is also possible to express the recombinant Ab fragment without being fused to another protein, molecules can be obtained which are principally suitable for a wide range of applications, including administration *in vivo*.

The affinities of antibody fragments derived from phage display libraries of the rearranged V-genes from immunised mice appear to be comparable with conventional mAb obtained after inducing secondary immune responses. However, if phage display libraries are employed which have not been generated from immunized human donors or animals ("naive" libraries), the affinities exhibited by scFv reagents typically fall in the range  $10^6 - 10^7 \text{ M}^{-1}$ , which is characteristic of primary immune responses. Therefore, potentially suitable reagents require further diversification by random mutation or by "chain shuffling" [11-12]. However, such procedures requires the additional



step of library formation and reselection. Methods for affinity maturation *in vitro*, allowing phages to be selected from a pool of reagents with very similar affinity or dissociation kinetics, have already been described. But it is *a priori* not clear whether they will meet with success for any given target molecules,  
5 because the affinities must typically be enhanced by a factor of 100 or more.

Like Ab, TCR belong to the immunoglobulin superfamily of proteins, and their building blocks share considerable structural similarity with those of Ab. Two types of TCR exist, TCR $\alpha\beta$  and TCR $\gamma\delta$ . Each of the four chains consists of an  
10 N-terminal V domain ( $V_{\alpha,\beta,\gamma,\delta}$ ) and a C domain ( $C_{\alpha,\beta,\gamma,\delta}$ ). Like in Ab, V domains are also characterized by CDR, which are also designated CDR1, CDR2, and CDR3. Only very few attempts to generate recombinant reagents on the basis of TCR chains have been described [34-35]. This can be attributed to the difficulty of expressing these constructs in heterologous systems, which  
15 usually leads to the formation of insoluble inclusion bodies and no soluble protein [36-37]. These difficulties have so far also precluded the construction of phage libraries displaying TCR. Such libraries could e.g. be extremely useful for the recognition of MHC molecules complexed with tumour antigen-derived peptides or viral peptides.

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Phage antibody technology as described in (W092/01047) offers the ability to isolate human reagents with Ab like reactivity directly. In this application, it is demonstrated for the first time that such reagents  
25 against self-antigens can be isolated from phage libraries derived from, for example, nonimmunised sources and from libraries prepared by synthetic recombination of V-gene sequences, preferably recombination of VH with, DH and JH, and VL with JL sequences. This application shows  
30 that single libraries derived in a manner described below can act as a source of reagents directed towards both foreign and self antigens, opening up the prospect of a large, universal library to isolate antibodies or/and recombinant reagents to any molecular target. It was

disclosed in patent application W092/01047 that antibody fragments can be displayed on the surface of bacteriophage and that they will bind antigen.

- 5 It follows from the state of the art described above that advances are required in a number of areas to generate recombinant reagents with Ab- or TCR-like reactivity and suitable affinity and specificity. This is particularly obvious in the case of reagents which are intended to be used in a clinical setting. In particular, the areas needing improvement are the following:

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To this end, constructs were developed that allow the expression of TCR variable chains. Furthermore, these constructs serve the development of TCR and chimeric Ab/TCR libraries, which combine elements of both, the humoral and cellular immune responses, so that chimeric recombinant reagents  
15 containing an Ab V domain as well as a TCR V domain can easily be prepared. The diversity of the types of phage display libraries (so far only antibody based libraries) is thus greatly expanded (TCR diversity), making it more probable that reagents of high specificity and affinity against any desired target molecule can be found within one of the libraries.

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### **SUMMARY OF THE INVENTION**

This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant  
25 phages comprise a vector having a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant  
30 thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent from each of the recombinant phages.

- In one embodiment, the TCR recognition element comprises a variable fragment of the TCR, or mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the variable TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments.
- 10 In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to C $\alpha$ , C $\beta$ 1, C $\beta$ 2, C $\gamma$  or C $\delta$ .
- 15 In another embodiment, the immunoglobulin recognition element is an antibody comprising a variable domain. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment the heavy chain comprises one or more C $\mu$ 1 constant domains. As contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more C $\kappa$  (kappa) or C $\lambda$  (lambda) domains. In another embodiment, the variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.
- 20
- 25

This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for an Fv fragment. In one embodiment, the Fv fragment is a single chain Fv (scFv) fragment. In another

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embodiment the vector has a polynucleotide which codes for a Fab fragment.

Further as provided herein, the recombinant phages  
5 comprise a vector having a polynucleotide, which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof, wherein a linker region joins the TCR  
10 recognition element, and the Ig recognition element of the reagent. The linker region comprises a nucleic acid encoding an peptide or polypeptide which is characterized as: i) aiding in folding of the domains, ii) supporting the stabilization of the intact protein construct.

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Further as provided herein, the recombinant phages comprise a vector having a polynucleotide, which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an  
20 Ig recognition element, and/or a mutation and variant thereof, wherein a novel tag region joins the TCR recognition element, or the Ig recognition element of the reagent to gIII protein of the phage. The linker region comprises a nucleic acid encoding an peptide or  
25 polypeptide which is characterized as: i) aiding in protein purification and detection.

Further, in one embodiment, the vector comprises a nucleic acid which codes for a second molecule that is  
30 linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target. The second molecule enhances the overall avidity of the interaction of the reagent with the target molecule.



This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant  
5 phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant  
10 chimeric TCR recognition element/immunoglobulin recognition element reagent from each of the recombinant phages, such reagents, including mutants and/or variants thereof, include but are not limited to the following: a single chain TCRV $\alpha$ /VL, a single chain TCRV $\beta$ /VL, a single  
15 chain TCRV $\alpha$ /VH, a single chain TCRV $\beta$ /VH, a single chain VL/TCRV $\alpha$ , a single chain VL/TCRV $\beta$ , a single chain VH/TCRV $\alpha$ , a single chain VH/TCRV $\beta$ , a single chain TCRV $\gamma$ /VL, a single chain TCRV $\delta$ /VL, a single chain TCRV $\gamma$ /VH, a single chain TCRV $\delta$ /VH, a single chain  
20 VL/TCRV $\gamma$ , a single chain VL/TCRV $\delta$ , a single chain VH/TCRV $\gamma$ , and/or a single chain VH/TCRV $\delta$ , or mutants and/or variants thereof.

This invention provides a phage-display library for  
25 screening for target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and in which the vector expresses a  
30 recombinant TCR recognition element from each of the recombinant phages.

In one embodiment the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the variable TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ),

or TCR variable  $\delta$  (TCRV $\delta$ ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to C $\alpha$ , C $\beta$ 1, C $\beta$ 2, C $\gamma$  or C $\delta$ .

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Such phage displayed reagents include but are not limited to the following: a single chain TCRV $\alpha$ /TCRV $\alpha$ , a single chain TCRV $\beta$ /TCRV $\beta$ , a single chain TCRV $\gamma$ /TCRV $\gamma$ , a single chain TCRV $\delta$ /TCRV $\delta$ , a single chain TCRV $\alpha$ /TCRV $\beta$ , a single chain TCRV $\alpha$ /TCRV $\gamma$ , a single chain TCRV $\alpha$ /TCRV $\delta$ , a single chain TCRV $\beta$ /TCRV $\alpha$ , a single chain TCRV $\beta$ /TCRV $\gamma$ , a single chain TCRV $\beta$ /TCRV $\delta$ , a single chain TCRV $\gamma$ /TCRV $\alpha$ , a single chain TCRV $\gamma$ /TCRV $\beta$ , a single chain TCRV $\gamma$ /TCRV $\delta$ , a single chain TCRV $\delta$ /TCRV $\alpha$ , a single chain TCRV $\delta$ /TCRV $\beta$ , a single chain TCRV $\delta$ /TCRV $\gamma$  and/or a mutation and variant thereof.

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Further, in one embodiment the vector comprises a nucleic acid which codes for a second molecule that is linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target. The second

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molecule enhances the overall avidity of the interaction of the reagent with the target.

This invention provides a soluble recombinant chimeric  
5 TCR recognition element/Ig recognition element reagent.

In one embodiment the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one  
10 or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the variable TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains comprises one  
15 or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to C $\alpha$ , C $\beta$ 1, C $\beta$ 2, C $\gamma$  or C $\delta$ .

20 In another embodiment the immunoglobulin recognition element is an antibody comprising a variable domain. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment the  
25 heavy chain comprises one or more C $\mu$ 1 constant domains. As contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more C $\kappa$  (kappa) or C $\lambda$  (lambda) domains. In another embodiment, the  
30 variable domain comprises one or more of the CDR1, CDR2 or CDR3 segments.

This invention provides a reagent which comprises a single chain Fv fragment. In another embodiment, the reagent comprises a Fab fragment

5 Further, as provided herein, the reagent has a linker region which comprises a nucleic acid encoding a peptide or polypeptide characterized as: i) aiding in folding of the domains, ii) supporting the stabilization of the intact protein construct. The nucleic acid sequence of  
10 the linker region is set forth in Figure 1.

Further, as provided herein, the reagent has a tag region which comprises a nucleic acid encoding a peptide or polypeptide characterized as: i) aiding in protein  
15 purification and detection. The nucleic acid sequence of the tag region is set forth in Figure 1.

Further, in one embodiment, the reagent comprises a second molecule that is linked to the reagent. The second  
20 molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target. The second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target.

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For example, the second molecule includes but is not limited to: a molecule with antibody or TCR like reactivity, a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide,  
30 carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule contemplated herein is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant

thereof which exhibits low affinity to their respective target.

This invention provides a reagent which comprises the following soluble chimeric polypeptides: a single chain  
5 TCRV $\alpha$ /VL, a single chain TCRV $\beta$ /VL, a single chain TCRV $\alpha$ /VH, a single chain TCRV $\beta$ /VH, a single chain VL/TCRV $\alpha$ , a single chain VL/TCRV $\beta$ , a single chain VH/TCRV $\alpha$ , VH/TCRV $\beta$ , a single chain TCRV $\gamma$ /VL, a single  
10 chain TCRV $\delta$ /VL, a single chain TCRV $\gamma$ /VH, a single chain TCRV $\delta$ /VH, a single chain VL/TCRV $\gamma$ , a single chain VL/TCRV $\delta$ , a single VH/TCRV $\gamma$ , and/or a single chain VH/TCRV $\delta$ , or mutants and/or variants thereof.

15 The nucleic acids, which code for the chimeric reagents are discussed above.

This invention provides a soluble recombinant TCR  
20 recognition element reagent. In one embodiment the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  
25  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the variable TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the  
30 TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to C $\alpha$ , C $\beta$ 1, C $\beta$ 2, C $\gamma$  or C $\delta$ .



This invention provides a reagent which comprises the following soluble TCR polypeptides: See before a single chain TCRV $\alpha$ /TCRV $\alpha$ , a single chain TCRV $\beta$ /TCRV $\beta$ , a single chain TCRV $\gamma$ /TCRV $\gamma$ , a single chain TCRV $\delta$ /TCRV $\delta$ , a single chain TCRV $\alpha$ /TCRV $\beta$ , a single chain TCRV $\alpha$ /TCRV $\gamma$ , a single chain TCRV $\alpha$ /TCRV $\delta$ , a single chain TCRV $\beta$ /TCRV $\alpha$ , a single chain TCRV $\beta$ /TCRV $\gamma$ , a single chain TCRV $\beta$ /TCRV $\delta$ , a single chain TCRV $\gamma$ /TCRV $\alpha$ , a single chain TCRV $\gamma$ /TCRV $\beta$ , a single chain TCRV $\gamma$ /TCRV $\delta$ , a single chain TCRV $\delta$ /TCRV $\alpha$ , a single chain TCRV $\delta$ /TCRV $\beta$ , a single chain TCRV $\delta$ /TCRV $\gamma$  and/or a mutation and variant thereof.

15 This invention provides an expression vector comprising a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof. This invention provides a vector comprising a polynucleotide encoding the TCR and the Ig elements, fragments, domains and/or segments in a tail-to-head transcriptional orientation.

In one embodiment, the vector comprises a polynucleotide which encodes a TCR recognition element having a variable fragment of the TCR, mutant and variant thereof. The polynucleotide encoding the variable fragment includes but is not limited to: one or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the polynucleotide encodes one or more of the CDR1, CDR2 or CDR3 segments of the variable domain. In another embodiment the vector comprises a polynucleotide which

encodes a TCR recognition element having a constant fragment. The polynucleotide encodes a constant fragment of the TCR which includes but is not limited to: to  $C\alpha$ ,  $C\beta 1$ ,  $C\beta 2$ ,  $C\gamma$  or  $C\delta$ .

5

In another embodiment, the vector comprises a polynucleotide which encodes a variable domain of an antibody. In another embodiment, the vector comprises a polynucleotide which codes for a heavy chain and/or a light chain of the antibody. In another embodiment the vector comprises a polynucleotide which codes for one or more VH fragments. In another embodiment the vector comprises a polynucleotide which codes for one or more  $C_H 1$  constant domains of the variable fragment. In another embodiment the vector comprises a polynucleotide which codes for one or more VL fragments. In another embodiment the vector comprises a polynucleotide which codes for one or more  $C_k$  (kappa) or  $C_\lambda$  (lambda). In another embodiment, the vector comprises a polynucleotide which codes for one or more of CDR1, CDR2 or CDR3 segments of the variable segment.

Further, this invention provides a vector comprising a polynucleotide which codes for an Fv fragment. In one embodiment the Fv fragment is a scFv fragment. In another embodiment, the vector has a polynucleotide which codes for a Fab fragment

Further as provided herein, the vector comprises a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof; and a polynucleotide of a

linker region which when expressed joins the T-cell receptor (TCR) recognition element, and the Ig recognition element of the reagent. The polynucleotide of the linker region comprises a nucleic acid having a  
5 sequence as set forth in Fig.1.

Further, as provided herein, the reagent has a tag region which comprises a nucleic acid encoding a peptide or polypeptide characterized as: i) aiding in protein  
10 purification and detection. The nucleic acid sequence of the tag region is set forth in Figure 1.

Further, in one embodiment of the invention, the vector comprises a polynucleotide which codes for a second  
15 molecule that is linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target. The second molecule enhances the overall avidity of the interaction of the reagent with  
20 the target molecule or a multimeric target.

For example, the second molecule includes but is not limited to: a molecule with Ab-or TCR like reactivity, a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme,  
25 single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule contemplated herein is a reagent directed against a specific MHC/peptide complex  
30 coupled to CD8, or variant thereof which exhibits low affinity to their respective target.

This invention provides a vector comprising a polynucleotide which codes for TCR recognition element,

and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/Ig recognition element reagent from each of the recombinant phages, such vectors comprise a polynucleotide encoding: a single chain TCRV $\alpha$ /VL, a single chain TCRV $\beta$ /VL, a single chain TCRV $\alpha$ /VH, a single chain TCRV $\beta$ /VH, a single chain VL/TCRV $\alpha$ , a single chain VL/TCRV $\beta$ , a single chain VH/TCRV $\alpha$ , VH/TCRV $\beta$ , a single chain TCRV $\gamma$ /VL, a single chain TCRV $\delta$ /VL, a single chain TCRV $\gamma$ /VH, a single chain TCRV $\delta$ /VH, a single chain VL/TCRV $\gamma$ , a single chain VL/TCRV $\delta$ , a single VH/TCRV $\gamma$ , and/or a single chain VH/TCRV $\delta$ , or mutants and/or variants thereof.

This invention provides a method for creating a phage display chimeric TCR/Ig reagent comprising the steps of: obtaining a sample of cells; preparing mRNA of the cells, reverse transcribing mRNA of the cell population into cDNA sequences of TCR and Ig; amplifying the cDNA; cloning the population of DNA fragments into expression vectors; providing nucleic acid expression vectors which are capable of being packaged; combining (i) a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population first component part of the TCR elements with (ii) a genetically diverse repertoire of nucleic acid sequences which encodes a unique or genetically diverse population of the immunoglobulin elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR and Ab polypeptides; also with

the property of binding specifically to a target molecule of interest; expressing said library from said vectors in recombinant host organism cells, each of the said polypeptide chain components being expressed as a  
5 recombinant chimeric protein on its own or as part of phage particles which are components of the library; selecting from said expressed library by binding to a target molecule of interest said reagents binding specifically to the target molecule, thereby  
10 producing a recombinant chimeric TCR /Ig reagent.

This invention provides a method for creating a phage display TCR reagent comprising the steps of: obtaining a sample of cells; preparing mRNA of cells; reverse  
15 transcribing mRNA of the cell population into cDNA sequences of T-cell receptor; amplifying the cDNA; cloning the population of DNA fragments into expression vectors; providing nucleic acid expression vectors which are capable of being packaged; combining a genetically  
20 diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population of the TCR elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR polypeptide; also with the property of binding  
25 specifically to a target molecule of interest; expressing said library from said vectors in recombinant host organism cells, said polypeptide chain components being expressed as a recombinant TCR protein on its own or or as part of phage particles which are components of the  
30 library; selecting from said expressed library by binding to a target molecule of interest said reagents binding specifically to the target molecule, thereby producing a recombinant TCR reagent.



This invention provides a method for selecting recombinant reagents directed against a molecular target, said method comprising: contacting the phage display chimeric library or the phage display TCR library as  
5 hereinabove described, with the target molecule so as to form a complex, dissociating the specifically bound phage from the complex; amplifying the rescued phage in a bacterial host; repeating binding, dissociation and amplification steps; screening said library on a target  
10 molecule. In another embodiment, the method further comprises characterizing the the selected phage particles and their respective reagents.

15 The target is any composition: molecule, a complex, a nucleic acid sequence, a polypeptide, peptide fragment or any composition that can be assayed for its ability to function in given capacity or compound. The target molecule perhaps synthetic, recombinant or biological  
20 sample.

This invention provides a method for diagnosing a subject with a pathogenic condition for e.g. a malignant disease comprising the steps of: a) obtaining a sample from the  
25 subject, b) contacting the sample with a recombinant reagent as discussed above, wherein the reagent is specific for a specific target molecule so as to form a complex, c) detecting the complex, the presence of which is indicative of the subject having the disorder.

30

This invention provides use of a pharmaceutical composition comprising the reagent as described above, for the prevention or treatment of an infectious or autoimmune diseases, selected from the group consisting

of ankylosing spondylitis, Reiter's disease, psoriatic spondylitis, psoriasis vulgaris and Behcet disease, and rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, systemic lupus erythematosus, Sjogren's disease, IDDM, Addison disease, Graves disease, Hashimoto disease, coeliac disease, primary biliary cirrhosis, pemphigus vulgaris, epidermolysis bullosa acquisita, Hodgkin's disease, multiple sclerosis, optic neuritis, narcolepsy, myasthenia gravis, Goodpasture syndrome and alopecia areata.

This invention provides a method of treating a subject with a disease or a pathogenic conditions, comprising administering to the subject an effective amount of the reagent as described above, thereby treating the subject with the disease or pathogenic condition.

This invention provides a method for imaging a pathological condition for e.g. a neoplastic disorder in a subject comprising the steps of administering to the subject an amount of the recombinant reagent as described above, wherein the reagent is labeled, and detecting the label.

## **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1A. The phagmid vector used for cloning the libraries. It contains M13 origin of replication and plasmid origin of replication (M13 and colE1, respectively), ribosome binding site (RBS), hatch box includes six amino acids from 5'end of C domain, His and myc tags flanked by amber stop codon (amb), followed by DNA encoding the gIII protein of the bacteriophage. The expression is under the control of LacZ promoter. The linker between XhoI and SalI/ApaI contains eight amino acids from the 5'end of the C domain followed by the classical (Gly4Ser)3 linker.

Figure 1B. The various assembly combinations of the current construct libraries are listed.

Figure 2: The primers used for characterization are indicated

5

Figure 3A. Representative Phage ELISA of TCRV $\alpha$   $N_L$  clones. Phage supernatants were detected by: Anti-M13 (Rows A,B), Protein A-HRP (C,D) and Protein L-HRP (E,F). The figure depicts the gray scale analysis of ELISA OD450-650.

10

Figure 3B. Representative Phage ELISA of TCRVH/V $\beta$  clones. Phage supernatants were detected by: Anti-M13 (rows A,B), Protein A-HRP (C,D) and Protein L-HRP (E,F). The figure depicts the gray scale analysis of ELISA OD450-650. Column 7 is positive control from the Tomlison library containing DP47 (VH3) and DPK12.

15

Figure 3C. ELISA analysis of soluble single chain chimeric TCR clones. Number indicated ELISA OD450-650. Periplasmic extract prepared from HB2151 infected clones were detected by Protein A-HRP and Protein L-HRP.

20

Figure 4. Mutations in TCR molecule.

Figure 5: The linker and tag sequences and position in the phagmid vector is indicated

25

Figure 6: Nucleic sequence of the oligonucleotides used to amplify the TCRV $\alpha$  gene segments. Degenerated nucleotides are underline, and NcoI and NotI restriction site are in bold. M= A/C, V= A/G/C, R= A/G, H= A/C/T, W= A/T, D= A/G/T, S= G/C, B= G/C/T, Y= C/T, N=A/C/G/T, K= G/T. TCRV $\alpha$  genes in brackets indicates that these similar segments can also be amplified by the indicated degenerate primer. C $\alpha$ -For-XhoI is the 3' oligonucleotide that aneal to the 5'end of the C $\alpha$  gene segment.

30

Figure 7. Nucleic sequence of the primers used to amplify the TCRV $\beta$  genes. Degenerated nucleotides are underline. Sall and NotI restriction sites are indicated by bold letters. M= A/C, V= A/G/C, R= A/G, H= A/C/T, W= A/T, D= A/G/T, S= G/C, B= G/C/T, Y= C/T, N=A/C/G/T, K= G/T. TCRV genes that can be amplified by the same  
 5 degenerated primers are listed in brackets. The 3' primers were designed to specifically anneal to C $\beta$ 1 and C $\beta$ 2 gene segment of TCR.

Figure 8. Sample sequencing of the various libraries.

10 Figure 9. Sequence analysis of clones originated from TG-1 bacteria infected by phage.

Figure 10. Fig10 shows sequence sampling of some of these selections.

## 15 DETAILED DESCRIPTION OF THE INVENTION

This invention provides a chimeric phage display library, TCR phage display library, recombinant chimeric reagents, methods of making the libraries methods for identifying recombinant reagents, oligonucleotides, linkers, tags, methods of purification, methods of increasing the avidity of recombinant  
 20 reagents, methods of diagnosing and treating a subject with a disease or a pathogenic condition.

The scTCR and scTCR-Ig molecules as provided herein are extremely  
 25 versatile, both for diagnosis as well as for treatment of various diseases, and will substantially complement the battery of diagnostically and therapeutically applicable reagents. Furthermore, scTCR and scTCR-Ig molecules have an important application as diagnostic reagents for other pathological conditions like CD8, like urine or serum, since they are very important diagnostic  
 30 markers e.g. in transplantation (transplant rejection) or for some diseases (e.g. nephrological problems), respectively. Moreover, use of such reagents could help e.g. to study the expression of peptide-devoid or peptide-filled HLA molecules in different lymphoid organs and cell subsets, since, the

mechanisms governing positive selection of T cell are up till now not understood.

Further, this invention provides therapy of malignant diseases using labeled or  
5 unlabeled constructs, eradication of infections by eliminating the infectious  
agents with labeled or unlabeled constructs, treatment of autoimmune  
diseases, and detection of molecules within cells.

10 I. Chimeric phage-display and TCR phage-display  
libraries

This invention provides chimeric phage-display,  
comprising a plurality of recombinant phages, wherein  
each of the recombinant phages comprise a vector having a  
15 polynucleotide which codes for a TCR recognition element,  
and/or a mutation and variant thereof; and a  
polynucleotide which codes for an Ig recognition element,  
and/or a mutation and variant thereof, and in which the  
vector expresses a recombinant chimeric TCR recognition  
20 element/immunoglobulin recognition element reagent from  
each of the recombinant phages.

This invention provides TCR phage-display libraries,  
comprising a plurality of recombinant phages, wherein  
25 each of the recombinant phages comprise a vector having a  
polynucleotide which codes for a TCR recognition element,  
and/or a mutation and variant thereof; and in which the  
vector expresses a recombinant TCR recognition element  
from each of the recombinant phages.

30

The "Ig recognition element" includes heavy chain and  
light chain variable domain (Ig-VH and Ig-VL) and natural  
or partly or wholly synthetically produced protein. The  
term also covers any protein having a binding domain



which is homologous to an immunoglobulin binding domain. These proteins can be derived from natural sources, or partly or wholly synthetically produced "Ig homologs" in this application include members of the Ig superfamily  
5 and homologs thereof. "Homolog" means that the polypeptides having the same or conserved residues at a corresponding position in their primary, secondary or tertiary structure. The term also extends to two or more nucleotide sequences encoding the homologous  
10 polypeptides.

A "domain" is a part of a protein that is folded within itself and independently of other parts of the same protein and independently of a complementary binding  
15 member.

As defined herein, a "library" is a collection of nucleotide sequences, e.g. DNA, within clones; or a genetically diverse collection of polypeptides, or  
20 specific binding pair members, or polypeptides displayed on phages capable of selection or screening to provide an individual polypeptide or a mixed population of polypeptides.

25 The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of  
30 their heavy chains, antibodies can be assigned to different classes of which there are five major classes: IgA, IgD, IgE, IgG and IgM. Several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, and IgG4; IgA1 and IgA2. The term "antibody

and antibody fragments" is used in the present specification and claims in the broadest sense and specifically covers single monoclonal antibodies as well as antibody fragments (e.g., Fab, F(ab')<sub>2</sub>, Fv and scFv),  
5 as long as they specifically recognize a molecular target. The invention thus relates to methods according to the invention wherein the antibody fragment is selected from the group consisting of Fab, F(ab')<sub>2</sub>, Fv, scFv and other molecular target-binding subsequences of  
10 an Ab.

This invention provides "mutants, variants and homologs" of the TCR and immunoglobulin polypeptides. Homolog means a polypeptides having the same or conserved  
15 residues at a corresponding position in their primary, secondary or tertiary structure. The term also extends to two or more nucleotide sequences encoding the homologous polypeptides. Examples for homologous peptides are the Ig isotypes. As contemplated herein, the TCR and/or Ig  
20 includes Ab or polypeptide components thereof, this is referring not only to diversity that can exist in the natural population of cells or organisms, but also diversity that can be created by artificial mutation *in vitro* or *in vivo*.

25

The polynucleotide comprises or encodes the Ig and the TCR includes RNA, cDNA, genomic DNA, fragments, isoforms, variants, mutants, alleles, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or  
30 derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates,

etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). Also included are synthetic molecules that  
5 mimic nucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule. Molecules substantially homologous to primary structural sequence but which include,  
10 e.g., *in vivo* or *in vitro* chemical and biochemical modifications or which incorporate unusual amino acids. The nucleic acid may be modified. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitinylation, labeling, e.g., with radionuclides, and various enzymatic modifications, as will be readily  
15 appreciated by those well skilled in the art.

Mutations can be introduced into a polynucleotide such that a particular codon is changed to a codon which codes for a different amino acid but the function is maintained. Such a mutation is generally made by making the  
20 fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a  
25 conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to another amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to  
30 alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid

belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Amino acids containing aromatic ring structures are phenylalanine, tryptophan, and tyrosine. The polar neutral amino acids  
5 include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such alterations will not be expected to affect apparent molecular weight as determined by polyacrylamide gel  
10 electrophoresis, or isoelectric point.

Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- 15 - Ser for Thr such that a free - hydroxyle group can be maintained; and
- Gln for Asn such that a free amino group can be maintained.

Synthetic DNA sequences allow convenient construction of genes which will express analogs or "muteins". A general method for site-specific incorporation  
20 of unnatural amino acids into proteins is described [Noren, et al. *Science*, 244:182-188 (April 1989)]. This method may be used to create analogs with unnatural amino acids.

A "nucleic acid" or "polynucleotide" refers to the phosphate ester polymeric  
25 form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in  
30 particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (e.g., restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA

molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to a mRNA). A "recombinant DNA" is a DNA that has undergone a molecular biological manipulation.

"Substantial identity" or "substantial sequence identity" mean that two sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap which share at least 65-99 percent sequence identity, share at least 75 percent sequence identity, share at least 80 percent sequence identity, share at least 90 percent sequence identity, preferably at least 95 percent sequence identity, more preferably at least 99 percent sequence identity or more. The following terms are used to describe the sequence relationships between two or more nucleic acid molecules or polynucleotides: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence.

Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. (USA)* 85:2444, or by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI).

30

Variant(s), as the term is used herein, are polynucleotides or polypeptides that differ from a reference polynucleotide or polypeptide respectively. Variants in this sense are described below and elsewhere in the present disclosure in greater detail. (1) A polynucleotide that differs in nucleotide



sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may be silent, i.e., they may not alter the amino acids encoded by the polynucleotide. Where alterations are limited to silent changes of this type a variant will encode a polypeptide with the same amino acid sequence as the reference polypeptide. Changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. (2) A polypeptide that differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference and the variant are closely similar overall and, in many region, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination. (3) A variant may also be a fragment of a polynucleotide or polypeptide of the invention that differs from a reference polynucleotide or polypeptide sequence by being shorter than the reference sequence, such as by a terminal or internal deletion. A variant of a polypeptide of the invention also includes a polypeptide which retains essentially the same biological function or activity as such polypeptide, e.g., proproteins which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide. (4) A variant may also be (i) one in which one or more of the amino acid residues are substituted with a conserved or non- conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. (5) A variant of the polynucleotide or polypeptide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that

is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by organic chemical synthesis, by mutagenesis techniques, including those applied to polynucleotides, cells or organisms, or may be made by recombinant means. Among polynucleotide variants in this regard are variants that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non- coding regions or both. Alterations in the coding regions may produce conservative or non- conservative amino acid substitutions, deletions or additions. All such variants defined above are deemed to be within the scope of those skilled in the art from the teachings herein and from the art.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package

(Devereux, J., et al., Nucleic Acids Research 12(l): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., J. Molec. Biol. 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity. Preferred parameters for polypeptide sequence comparison include the following:

- 1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970 )
- Comparison matrix: matches = + 10, mismatch = 0 Gap Penalty: 50 Gap Length Penalty: 3 Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons. Preferred polymicleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence as set forth in Figures 5, 6, and/or 7 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides as set forth in Figures 5,6, and/or 7 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides; in Figures 5,6, and/or 7, or:  $nn - (xn \cdot y)$ , wherein  $nn$  is the number of nucleotide alterations,  $xn$  is the total number of nucleotides in SEQ ID NO: 1,  $y$  is 0.50 for 50 %, 0.60 for 60 %, 0.70 for 70 %, 0.80 for 80 %, 0.85 for 85 %, 0. 90 for 90 %, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\cdot$  is the symbol for the multiplication operator, and wherein any non-integer product of  $xn$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $xn$ , Alterations of a polynucleotide sequence encoding the polypeptide may create

nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polymucleotide following such alterations.

- 5 The term "variable" refers to the fact that certain portions of the variable domains of Ig or TCR differ extensively in sequence among Ab or TCR and are used in the binding and specificity of each particular Ab or TCR for its particular molecular target. The variability is
- 10 concentrated in three segments called complementarity determining regions (CDR) or hypervariable regions both in the light chain and the heavy chain variable domains (Ig or TCR $\alpha$ , or  $\beta$ , or  $\gamma$ , or  $\delta$ ).
- 15 In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular
- 20 Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994)]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (1994)];
- 25 "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription and Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes"
- 30 [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

A "phage-display library" is a protein expression library, constructed in vectors, that expresses a

collection of cloned protein sequences as fusion with a phage coat protein. Thus, in the context of the invention, single-chain recombinant proteins having ligand-binding potential are expressed as fusion proteins on the exterior of the phage particle. This disposition advantageously allows contact and binding between the recombinant binding protein and a ligand. Those having ordinary skill in the art will recognize that phage clones expressing binding proteins specific for the ligand can be substantially enriched by serial rounds of phage binding to the ligand, especially when this is immobilised, dissociated from the ligand and amplified by growth of rescued phage in bacterial host cells. The phage may be a filamentous phage. The host may be E.coli.

15

As provided herein, "phage" may be a replicable genetic display package in which the particle is displaying a member of a specific binding pair at its surface. The package may be a bacteriophage which displays a binding domain at its surface. As provided herein, the binding domain on the surface is a chimeric TCR/Ig and/or a TCR and includes domains, fragments, subdomains, which may be connected by a synthetic linker.

25 "TCR recognition element" comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In one embodiment, the TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains comprise one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment, the TCR



recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to  $C\alpha$ ,  $C\beta 1$ ,  $C\beta 2$ ,  $C\gamma$  or  $C\delta$ .

- 5 "The Ig recognition element" is an Ab. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment, the heavy chain comprises one or more  $C_H1$  constant domains. As  
10 contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more  $C_k$  (kappa) or  $C_\lambda$  (lambda) domains. In another embodiment, the variable domain comprises one or more of CDR1, CDR2 or  
15 CDR3 segments.

- This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phage, wherein each of the recombinant phages  
20 comprise a vector having a polynucleotide which codes for an Fv fragment. In one embodiment, the Fv fragment is a single chain Fv fragment. In another embodiment, the vector includes a polynucleotide which codes for a Fab fragment

25

- Further as provided herein, the recombinant phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig  
30 recognition element, and/or a mutation and variant thereof, wherein a linker region joins the TCR recognition element, and the Ig recognition element of the reagent. The linker region comprises a nucleic acid which is characterized as: i) aiding in folding of the



domains, ii) supporting the stabilization of the intact protein construct; wherein said nucleic acid has a sequence as set forth in Figure 1. Based on the information provided herein, one skilled in the art can  
5 make other linker sequences which aid in folding of the domains, and support the stabilization of the intact protein construct. In another embodiment, the linker comprises a nucleic acid having the sequence as set forth in Figure 5, including mutations, homologs, and variants  
10 thereof.

As shown herein, the novel linkers which were developed aid in folding of the domains, and support the stabilization of the intact protein construct, allowing for the first time the expression of scTCR protein constructs consisting only of  
15 two TCR V-domains on the surface of phage particles from a phage display library. The linkers contain a small number of the N-terminal amino acids of a constant TCR domains followed by the conventional linker sequence (Gly4Ser)3. The novel linker has the additional advantage that the corresponding DNA sequence is complementary to the two primers allowing  
20 universal amplification of the respective TCR gene.

Furthermore, the novel linker provides a means to purify and detect the recombinant protein in an entirely novel way. The junctional regions between the C-terminal (Gly4Ser)3 and any of the two N-terminally located TCR  
25 C-domain-derived peptides provide novel epitopes for reagents like Ab, which can recognize them with high specificity and affinity. Such reagents are not expected to react with TCR molecules on T cells, since these do not contain the junctional region between the peptides. The linkers should also be extremely useful tools to detect phage particles carrying recombinant proteins.  
30 Finally, it is contemplated herein, that the TCR C-domain-derived sequences are combined not only with the conventional (Gly4Ser)3 linker, but with other linking sequences as well, e.g. to provide recognition elements for effector molecules.

Further as provided herein, the novel tag which were developed contain a small number of the N-terminal amino acids of a constant TCR or Ig domains followed by the conventional his myc tags. The novel tag has the additional  
5 advantage that the corresponding DNA sequence is complementary to the two primers allowing universal amplification of the respective TCR or Ig gene. The tag should also be extremely useful tools to detect phage particles carrying recombinant proteins. Finally, it is contemplated herein, that the TCR C-domain-derived sequences are combined not only with the conventional his  
10 myc tags, but with other sequences as well, e.g. to provide elements for effector molecules.

The production of recombinant reagents against a particular MHC/peptide  
15 complex is provided herein. This interaction could be stabilized by linking another molecule with reactivity towards invariant parts of the MHC class I molecule e.g. CD8, to the first reagent, thereby increasing the avidity. However, care would have to exercised to prevent reactivity of these reagents on their own, because this would lead to the recognition of all HLA class I  
20 molecules by the construct. Therefore, the CD8 molecule would have to be mutated as to reduce its affinity towards MHC class I molecules. Since the structure of an HLA class I/CD8 complex has been solved [56], the interacting amino acids are precisely known, and decreasing the affinity of the interaction by site-directed mutagenesis will be simple.

25 For example, the the second molecule includes but is not limited to: a molecule with antibody or TCR like reactivity, a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or  
30 inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule contemplated herein is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant

thereof which exhibits low affinity to their respective target.

#### A. Phage Display Libraries

5 This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation  
10 and variant thereof; and a polynucleotide which codes for an (Ig) recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/Ig recognition element reagent from each of the recombinant  
15 phages, such reagents, including mutants and/or variants thereof, include but are not limited to the following: a single chain TCRV $\alpha$ /VL, a single chain TCRV $\beta$ /VL, a single chain TCRV $\alpha$ /VH, a single chain TCRV $\beta$ /VH, a single chain VL/TCRV $\alpha$ , a single chain VL/TCRV $\beta$ , a single chain  
20 VH/TCRV $\alpha$ , a single chain VH/TCRV $\beta$ , a single chain TCRV $\gamma$ /VL, a single chain TCRV $\delta$ /VL, a single chain TCRV $\gamma$ /VH, a single chain TCRV $\delta$ /VH, a single chain VL/TCRV $\gamma$ , a single chain VL/TCRV $\delta$ , a single chain VH/TCRV $\gamma$ , and/or a single chain VH/TCRV $\delta$ , or mutants and/or  
25 variants thereof.

In one embodiment, the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not  
30 limited to: one or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the variable TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ),

TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of  
5 the TCR includes but is not limited to C $\alpha$ , C $\beta$ 1, C $\beta$ 2, C $\gamma$  or C $\delta$ .

Such phage displayed reagents include but are not limited to the following: single chain TCRV $\alpha$ /TCRV $\alpha$ , a single  
10 chain TCRV $\beta$ /TCRV $\beta$ , a single chain TCRV $\gamma$ /TCRV $\gamma$ , a single chain TCRV $\delta$ /TCRV $\delta$ , a single chain TCRV $\alpha$ /TCRV $\beta$ , a single chain TCRV $\alpha$ /TCRV $\gamma$ , a single chain TCRV $\alpha$ /TCRV $\delta$ , a single chain TCRV $\beta$ /TCRV $\alpha$ , a single chain TCRV $\beta$ /TCRV $\gamma$ , a single chain TCRV $\beta$ /TCRV $\delta$ , a single chain TCRV $\gamma$ /TCRV $\alpha$ , a single  
15 chain TCRV $\gamma$ /TCRV $\beta$ , a single chain TCRV $\gamma$ /TCRV $\delta$ , a single chain TCRV $\delta$ /TCRV $\alpha$ , a single chain TCRV $\delta$ /TCRV $\beta$ , a single chain TCRV $\delta$ /TCRV $\gamma$  and/or a mutation and variant thereof.

## II. Reagents:

20 This invention provides a recombinant chimeric TCR recognition element/ Ig recognition element reagent. In the preferred embodiment, the reagent is soluble.

In one embodiment, the TCR recognition element comprises  
25 a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the variable TCR  
30 variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another

embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to  $C\alpha$ ,  $C\beta 1$ ,  $C\beta 2$ ,  $C\gamma$  or  $C\delta$ .

- 5 In another embodiment the Ig recognition element is an antibody comprising a variable domain. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment the heavy
- 10 chain comprises one or more  $C_H1$  constant domains. As contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more  $C_k$  (kappa) or  $C_\lambda$  (lambda) domains. In another embodiment, the
- 15 variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.

This invention provides a reagent which comprises a single chain Fv fragment. In another embodiment the

20 reagent comprises a Fab fragment

Further, as provided herein, the reagent has a linker region comprises a nucleic acid which is characterized as: i) aiding in folding of the domains, ii) supporting

25 the stabilization of the intact protein construct. The nucleic acid sequence of the linker region was described above. Further, in one embodiment the reagent comprises a second molecule that is linked to the reagent. The second molecule is a molecule which interacts with a

30 second, nonoverlapping determinant of the target molecule or a multimeric target. The second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target with the proviso that the affinity of the second molecule to its

interaction site is in itself insufficient to allow interaction with the target molecule or multimeric target under physiologic conditions.

- 5 For example, the second molecule includes but is not limited to: a molecule with antibody or TCR like reactivity, a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or  
10 inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule contemplated herein is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant thereof which exhibits low affinity to their respective  
15 target.

This invention provides a reagent which comprises the following soluble chimeric polypeptides: a single chain TCRV $\alpha$ /VL, a single chain TCRV $\beta$ /VL, a single chain  
20 TCRV $\alpha$ /VH, a single chain TCRV $\beta$ /VH, a single chain VL/TCRV $\alpha$ , a single chain VL/TCRV $\beta$ , a single chain VH/TCRV $\alpha$ , VH/TCRV $\beta$ , a single chain TCRV $\gamma$ /VL, a single chain TCRV $\delta$ /VL, a single chain TCRV $\gamma$ /VH, a single chain TCRV $\delta$ /VH, a single chain VL/TCRV $\gamma$ , a single chain  
25 VL/TCRV $\delta$ , a single VH/TCRV $\gamma$ , and/or a single chain VH/TCRV $\delta$ , or mutants and/or variants thereof. The nucleic acid which codes for the chimeric reagents are discussed above.

- 30 This invention provides a soluble recombinant TCR recognition element reagent. In one embodiment, the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment



includes but is not limited to: one or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment, the variable TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR  
5 variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domain comprise one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not  
10 limited to C $\alpha$ , C $\beta$ 1, C $\beta$ 2, C $\gamma$  or C $\delta$ .

This invention provides a reagent which comprises the following soluble TCR polypeptides: single chain TCRV $\alpha$ /TCRV $\alpha$ , a single chain TCRV $\beta$ /TCRV $\beta$ , a single chain  
15 TCRV $\gamma$ /TCRV $\gamma$ , a single chain TCRV $\delta$ /TCRV $\delta$ , a single chain TCRV $\alpha$ /TCRV $\beta$ , a single chain TCRV $\alpha$ /TCRV $\gamma$ , a single chain TCRV $\alpha$ /TCRV $\delta$ , a single chain TCRV $\beta$ /TCRV $\alpha$ , a single chain TCRV $\beta$ /TCRV $\gamma$ , a single chain TCRV $\beta$ /TCRV $\delta$ , a single chain TCRV $\gamma$ /TCRV $\alpha$ , a single chain TCRV $\gamma$ /TCRV $\beta$ , a single chain  
20 TCRV $\gamma$ /TCRV $\delta$ , a single chain TCRV $\delta$ /TCRV $\alpha$ , a single chain TCRV $\delta$ /TCRV $\beta$ , a single chain TCRV $\delta$ /TCRV $\gamma$  and/or a mutation and variant thereof.

In one embodiment the TCR recognition element comprises a  
25 variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the variable TCR variable  $\alpha$  (TCRV $\alpha$ ),  
30 TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains comprise one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the

TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to  $C\alpha$ ,  $C\beta 1$ ,  $C\beta 2$ ,  $C\gamma$  or  $C\delta$ .

- 5 In another embodiment the Ig recognition element is an Ab. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment the heavy chain comprises one or more CH1
- 10 constant domains. As contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more  $C\kappa$  (kappa) or  $C\lambda$  (lambda) domains. In another embodiment, the variable domain comprises one or more of
- 15 CDR1, CDR2 or CDR3 segments.

This invention provides a reagent which comprises a phage particle displaying single chain Fv fragment. In another embodiment the reagent comprises phage particle

20 displaying Fab fragment

This invention provides a phage display reagent which display the following soluble chimeric polypeptides: a single chain  $TCRV\alpha/VL$ , a single chain  $TCRV\beta/VL$ , a single

25 chain  $TCRV\alpha/VH$ , a single chain  $TCRV\beta/VH$ , a single chain  $VL/TCRV\alpha$ , a single chain  $VL/TCRV\beta$ , a single chain  $VH/TCRV\alpha$ , a single chain  $VH/TCRV\beta$ , a single chain  $TCRV\gamma/VL$ , a single chain  $TCRV\delta/VL$ , a single chain  $TCRV\gamma/VH$ , a single chain  $TCRV\delta/VH$ , a single chain

30  $VL/TCRV\gamma$ , a single chain  $VL/TCRV\delta$ , a single chain  $VH/TCRV\gamma$ , and/or a single chain  $VH/TCRV\delta$ , or mutants and/or variants thereof. The nucleic acid which codes for the chimeric reagents are discussed above.

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This invention provides a soluble recombinant TCR recognition element reagent. In one embodiment the TCR recognition element comprises a variable fragment of the  
5 TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the variable TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR  
10 variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains comprises one or more of CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited  
15 to C $\alpha$ , C $\beta$ 1, C $\beta$ 2, C $\gamma$  or C $\delta$ .

This invention provides phage display reagent which comprises the following soluble TCR polypeptides: single chain TCRV $\alpha$ /TCRV $\alpha$ , a single chain TCRV $\beta$ /TCRV $\beta$ , a single  
20 chain TCRV $\gamma$ /TCRV $\gamma$ , a single chain TCRV $\delta$ /TCRV $\delta$ , a single chain TCRV $\alpha$ /TCRV $\beta$ , a single chain TCRV $\alpha$ /TCRV $\gamma$ , a single chain TCRV $\alpha$ /TCRV $\delta$ , a single chain TCRV $\beta$ /TCRV $\alpha$ , a single chain TCRV $\beta$ /TCRV $\gamma$ , a single chain TCRV $\beta$ /TCRV $\delta$ , a single chain TCRV $\gamma$ /TCRV $\alpha$ , a single chain TCRV $\gamma$ /TCRV $\beta$ , a single  
25 chain TCRV $\gamma$ /TCRV $\delta$ , a single chain TCRV $\delta$ /TCRV $\alpha$ , a single chain TCRV $\delta$ /TCRV $\beta$ , a single chain TCRV $\delta$ /TCRV $\gamma$  and/or a mutation and variant thereof.

### III. Expression vectors:

30 This invention provides an expression vector comprising a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and polynucleotide

which codes for an Ig recognition element, and/or a mutation and variant thereof.

This invention provides for a replicable vector  
5 comprising the isolated nucleic acid molecule of the DNA virus. The vector includes, but is not limited to: a plasmid, cosmid, phage or yeast artificial chromosome (YAC) which contains at least a portion of the isolated nucleic acid molecule. As an example to obtain these  
10 vectors, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with DNA ligase. Alternatively, linkers can be ligated to the insert DNA which correspond  
15 to a restriction site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available and known to an ordinary skilled practitioner.

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20 The term "vector", refers to viral expression systems, autonomous self-replicating circular DNA (plasmids), and includes both expression and nonexpression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector," this includes both extrachromosomal circular DNA and DNA that has been incorporated into the  
25 host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome.

Expression vectors which can be used other than adenovirus include, but are  
30 not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus, swinepox virus, pox virus, rhabdovirus, herpes simplex virus, baculovirus, herpes simplex virus, adeno-associated virus, retrovirus, cytomegalovirus, human cytomegalovirus, papillomavirus, Epstein Barr virus (EBV), mouse mammary tumor virus (MMTV), Moloney

murine leukemia virus and plasmid and cosmid DNA vectors, to name but a few.

5 In one embodiment, the adenoviral vector is deficient in at least one essential gene function of the EI region of the adenoviral genome, particularly the Ela region, more preferably, the vector is deficient in at least one essential gene function of the EI region and part of the E3 region (e.g., an XbaI deletion of the E3 region) or, alternatively, the vector is deficient in at least one essential gene function of the EI region and at least one essential gene function of the  
10 E4 region. Adenoviral vectors deficient in at least one essential gene function of the E2a or E2b region and adenoviral vectors deficient in all of the E3 region also are contemplated here and are well known in the art. Furthermore, the viral vector's coat protein can be modified so as to incorporate a specific protein binding sequence.

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The term "plasmid" refers to an autonomous circular DNA molecule capable of replication in a cell, and includes both the expression and nonexpression types. Where a recombinant microorganism or cell culture is described as hosting an "expression plasmid", this includes latent viral DNA integrated into  
20 the host chromosome(s). Where a plasmid is being maintained by a host cell, the plasmid is either being stably replicated by the cells during mitosis as an autonomous structure or is incorporated within the host's genome.

Regulatory elements required for expression include promoter or enhancer  
25 sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA  
30 polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well-known in the art, for example the methods described above for constructing vectors in general. Enhancers were originally detected as genetic



elements that increased transcription from a promoter located at a distant position on the same molecule of DNA. This ability to act over a large distance had little precedent in classic studies of prokaryotic transcriptional regulation. Subsequent work showed that regions of DNA with enhancer activity are  
5 organized much like promoters. That is, they are composed of many individual elements, each of which binds to one or more transcriptional proteins.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals,  
10 terminators, and the like, that provide for the expression of a coding sequence in a host cell. A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the  
15 transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences)  
20 responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

25 An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the  
30 coding sequence. A nucleic acid sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the

correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start  
5 signal, such a start signal can be inserted in front of the gene.

Below is a list of viral promoters, cellular enhancers and inducible enhancers that may be used, which include but are not limited to the following: ventricular myocyte  
10 specific promoter, cytomegalovirus, human cytomegalovirus, inflammatory promoters, TNF promoter, Rous Sarcoma Virus, Prostate Specific Antigen, Probasin, Immunoglobulin Heavy Chain, Immunoglobulin Light Chain, T-Cell Receptor, HLA, Interferon, Interleukin-2,  
15 Interleukin-2 Receptor, MHC Class II, Actin, Muscle Creatine Kinase, Proalbumin (Transthyretin), Elastase I, Metallothionein, Collagenase, Albumin Gene, Fetoprotein, Globin, c-fos, c-Ha-ras, Insulin, Neural Cell Adhesion Molecule (NCAM), antirypole, 2B (TH2B) Histone, Muse or  
20 Type I Collagen, Glucose-Regulated Proteins (GRP94 and GRP78), Human Serum Amyloid A (SAA), Troponin I (TN I), Platelet-Derived Growth Factor, Duchenne Muscular Dystrophy, SV40, Polyoma, Retroviruses, Papilloma Virus, Hepatitis B Virus, or Gibbon Ape Leukemia Virus.

25

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

30

The basic distinction between enhancers and promoters is operational. An enhancer region as a whole must be able to stimulate transcription at a distance; this need not be true of a promoter region or its component elements. On the other hand, a promoter must have one or more elements

that direct initiation of RNA synthesis at a particular site and in a particular orientation, whereas enhancers lack these specificities. Promoters and enhancers are often overlapping and contiguous, often seeming to have a very similar modular organization.

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A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to  
10 be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front  
15 of the gene.

In one embodiment the vector comprises a polynucleotide which encodes a TCR recognition element having a variable fragment of the TCR, mutant and variant thereof. The  
20 polynucleotide encoding the variable fragment includes but is not limited to: one or more of TCR variable  $\alpha$  (TCRV $\alpha$ ), TCR variable  $\beta$  (TCRV $\beta$ ), TCR variable  $\gamma$  (TCRV $\gamma$ ), or TCR variable  $\delta$  (TCRV $\delta$ ) domains. In another embodiment the polynucleotide encodes one or more of the  
25 complementarity determining residues (CDR) 1, CDR2 or CDR3 segments of the variable domain. In another embodiment the vector comprises a polynucleotide which encodes a TCR recognition element having a constant fragment. The polynucleotide encodes a constant fragment  
30 of the TCR includes but is not limited to: to C $\alpha$ , C $\beta$ 1, C $\beta$ 2, C $\gamma$  or C $\delta$ .

In another embodiment the vector comprises a polynucleotide which encodes a variable domain of an antibody. In another embodiment, the vector comprises a polynucleotide which codes for a heavy chain and/or a light chain of the antibody. In another embodiment the vector comprises a polynucleotide which codes for one or more heavy chain variable fragments (VH). In another embodiment the vector comprises a polynucleotide which codes for one or more C<sub>H</sub>1 constant domains of the variable fragment. In another embodiment the vector comprises a polynucleotide which codes for one or more light chain variable fragments (VL). In another embodiment the vector comprises a polynucleotide which codes for one or more C<sub>k</sub> (kappa) or C<sub>λ</sub> (lambda) domains. In another embodiment the vector comprises a polynucleotide which codes for one or more of CDR1, CDR2 or CDR3 segments of the variable segment.

Further, this invention provides a vector comprising a polynucleotide which codes for a Fv fragment. In one embodiment the Fv fragment is a single chain Fv fragment. In another embodiment the vector has a polynucleotide which codes for a Fab fragment

Further as provided herein, the vector comprises a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof; and a polynucleotide of a linker region which when expressed join the T-cell receptor (TCR) recognition element, and the immunoglobulin (Ig) recognition element of the reagent.

The polynucleotide of the linker region comprises a nucleic acid having a sequence as follows:

Further, in one embodiment of the invention, the vector  
5 comprises a polynucleotide which codes for a second molecule that is linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target. The second molecule enhances the  
10 overall avidity of the interaction of the reagent with the target molecule or a multimeric target.

For example, the second molecule includes but is not limited to: a molecule with antibody or TCR like  
15 reactivity, a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule  
20 contemplated herein is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant thereof which exhibits low affinity to their respective target.

25 This invention provides a vector comprising a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a  
30 mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent from each of the recombinant phages, such vectors comprise a polynucleotide encoding: a single chain TCRV $\alpha$ /VL, a



single chain TCRV $\beta$ /VL, a single chain TCRV $\alpha$ /VH, a single chain TCRV $\beta$ /VH, a single chain VL/TCRV $\alpha$ , a single chain VL/TCRV $\beta$ , a single chain VH/TCRV $\alpha$ , VH/TCRV $\beta$ , a single chain TCRV $\gamma$ /VL, a single chain TCRV $\delta$ /VL, a single chain  
5 TCRV $\gamma$ /VH, a single chain TCRV $\delta$ /VH, a single chain VL/TCRV $\gamma$ , a single chain VL/TCRV $\delta$ , a single VH/TCRV $\gamma$ , and/or a single chain VH/TCRV $\delta$ , or mutants and/or variants thereof.

10 This invention provides a vector comprising a polynucleotide encoding the T-cell receptor (TCR) and the immunoglobulin elements, fragments, domains and/or segments in a tail-to-head transcriptional orientation. In one embodiment the vector comprises transcription and  
15 translation control sequences. In another embodiment the vector comprises transcription control sequence which is selected from the group consisting of a promoter, an RNA polymerase initiation site, an RNA polymerase termination site, a TATA box, a CAT box, a poly A  
20 addition site, an enhancer and a part or combination thereof. In another embodiment, this invention provides the translation control sequence which are selected from the group consisting of a ribosome binding site, a leader sequence and a part or combination thereof.

25

Further, the vector comprises but is not limited to a reported gene. The following reporter genes must be used: luciferase,  $\beta$ -galactosidase, or  $\beta$ -lactamase. Other reporter genes include but are not limited to:  $\beta$ -lactamase and other antibiotic resistant gene, a cell surface marker as MHC I  
30 or II sub-types, a receptor for growth factor or cell adhesion and any gene of interest for therapeutic reasons. Additionally, the vector may comprise a marker inserted may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The